SAA (Serum Amyloid A): A Novel Predictor of Stroke-Associated Infections

Schweizer, Juliane ; Bustamante, Alejandro ; Lapierre-Fétaud, Vanessa ; Faura, Júlia ; Scherrer, Natalie ; Azurmendi Gil, Leire ; Fluri, Felix ; Schütz, Valerie ; Luft, Andreas ; Boned, Sandra ; Sanchez, Jean-Charles ; Montaner, Joan ; Katan, Mira

Abstract: BACKGROUND AND PURPOSE The aim of this study was to evaluate and independently validate SAA (serum amyloid A)-a recently discovered blood biomarker-to predict poststroke infections. METHODS The derivation cohort (A) was composed of 283 acute ischemic stroke patients and the independent validation cohort (B), of 367 patients. The primary outcome measure was any stroke-associated infection, defined by the criteria of the US Centers for Disease Control and Prevention, occurring during hospitalization. To determine the association of SAA levels on admission with the development of infections, logistic regression models were calculated. The discriminatory ability of SAA was assessed, by calculating the area under the receiver operating characteristic curve. RESULTS After adjusting for all predictors that were significantly associated with any infection in the univariate analysis, SAA remained an independent predictor in study A (adjusted odds ratio, 1.44 [95% CI, 1.16-1.79]; P=0.001) and in study B (adjusted odds ratio, 1.52 [1.05-2.22]; P=0.028). Adding SAA to the best regression model without the biomarker, the discriminatory accuracy improved from 0.76 (0.69-0.83) to 0.79 (0.72-0.86; P<0.001; likelihood ratio test) in study A. These results were externally validated in study B with an improvement in the area under the receiver operating characteristic curve, from 0.75 (0.70-0.81) to 0.76 (0.71-0.82; P<0.038). CONCLUSIONS Among patients with ischemic stroke, blood SAA measured on admission is a novel independent predictor of infection after stroke. SAA improved the discrimination between patients who developed an infection compared with those who did not in both derivation and validation cohorts. Registration: URL: https://www.clinicaltrials.gov. Unique identifier: NCT00390962.

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SAA (Serum amyloid A)

A Novel Predictor of Stroke Associated Infections

Juliane Schweizer, MD[1*], Alejandro Bustamante, MD, PhD[2,3*], Vanessa Lapierre-Fétaud, MSc[4], Júlia Faura, MSc[2], Natalie Scherrer[1], Leire Azurmendi Gil, PhD[4]; Felix Fluri, MD[5], Valerie Schütz, MD[1], Andreas Luft, MD[1], Sandra Boned, MD[3], Jean-Charles Sanchez, PhD[4**], Joan Montaner, MD, PhD[2**], Mira Katan, MD, MS[1**]

*Equally contributing first authors. **Equally contributing last authors.

Author Affiliations

[1]Department of Neurology, University Hospital Zurich, Switzerland; [2]Neurovascular Research Laboratory, Vall d'Hebron Institute of Research (VHIR), Universitat Autònoma de Barcelona, Spain; [3]Department of Neurology, Hospital Universitari Vall d'Hebron, Barcelona, Spain; [4]Translational Biomarker Group, Department of Human Protein Sciences, University of Geneva, Switzerland; [5]Department of Internal Medicine and Research, University Hospital Wuerzburg, Germany.

Short title: Serum amyloid A predicts post stroke infections

Contact information for the corresponding author

Alejandro Bustamante, MD, PhD

Neurovascular Research Laboratory

Vall d'Hebron Institute of Research (VHIR), Universitat Autònoma de Barcelona

0034 93 489 40 29

alejandro.bustamante@vhir.org

@VHIR_

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Abstract

Background and Purpose: The aim of this study was to evaluate and independently validate SAA (serum amyloid A)—a recently discovered blood biomarker—to predict poststroke infections.

Methods: The derivation cohort (A) was composed of 283 acute ischemic stroke patients and the independent validation cohort (B), of 367 patients. The primary outcome measure was any stroke-associated infection, defined by the criteria of the US Centers for Disease Control and Prevention, occurring during hospitalization. To determine the association of SAA levels on admission with the development of infections, logistic regression models were calculated. The discriminatory ability of SAA was assessed, by calculating the area under the receiver operating characteristic curve.

Results: After adjusting for all predictors that were significantly associated with any infection in the univariate analysis, SAA remained an independent predictor in study A (adjusted odds ratio, 1.44 [95% CI, 1.16–1.79]; *P*=0.001) and in study B (adjusted odds ratio, 1.52 [1.05–2.22]; *P*=0.028). Adding SAA to the best regression model without the biomarker, the discriminatory accuracy improved from 0.76 (0.69–0.83) to 0.79 (0.72–0.86; *P*<0.001; likelihood ratio test) in study A. These results were externally validated in study B with an improvement in the area under the receiver operating characteristic curve, from 0.75 (0.70–0.81) to 0.76 (0.71–0.82; *P*<0.038).

Conclusion: Among patients with ischemic stroke, blood SAA measured on admission is a novel independent predictor of infection after stroke. SAA improved the discrimination between patients who developed an infection compared with those who did not in both derivation and validation cohorts.

Clinical Trial Registration Information-URL: NCT00390962

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Non-standard Abbreviations and Acronyms

CRP C-reactive protein

IL interleukin

IQR interquartile range

IS ischemic stroke

NRI net reclassification improvement

OR odds ratio

PCT procalcitonin

ROC receiver operating characteristic

SAA serum amyloid A

SAI stroke-associated infection

1. Introduction

Nosocomial infections are the most common complication after ischemic stroke (IS) with frequencies of 30%[1]. Several studies have shown an independent association between stroke-associated infections (SAIs) and poor functional outcome, in particular, poststroke pneumonia as one of the primary risk factors of death after IS[2, 3]. The diagnosis of SAI is still challenging. Inflammatory markers, as well as clinical and radiological signs often occur after the onset of infection with a delay and therefore antibiotic treatment may be initiated also with a delay[4]. However, prophylactic antibiotic treatment in patients did not improve outcome[5, 6]. Consequently accurate and simply available predictive markers for better risk stratification and therapy guidance are needed. Several promising candidate blood biomarkers have been described recently to be independently associated with SAI[4, 7]. In a previous pilot study, an omics result- guided discovery approach, we could identify SAA (serum amyloid A; 1/2) as a potential prognostic biomarker of infection[8]. In the present study, we aimed to address the value of SAA in the prediction of SAI as compared with established inflammatory biomarkers in a derivation cohort within 72 hours after stroke onset (study A). Subsequently, we performed a more extensive validation study (study B) of the same marker measured at an earlier time-point, to determine the incremental value of this marker over other known clinical markers for the prediction of SAI.

2. Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

2.1 Ethics Statement

The studies (https://www.clinicaltrials.gov; Unique identifier: NCT00390962; PR[AG]157-2011) were conducted according to the principles expressed in the Declaration of Helsinki and

approved by the local ethics committees. All patients or their welfare guardians provided written informed consent for the collection of data, blood samples, and subsequent analyses.

2.2 Study Designs and Cohort Description

To evaluate and validate SAA to predict an infection after stroke, post hoc analysis of 2 separated prospective cohort studies was performed in 2 independent neurology departments.

For the **derivation study (**study A), patient eligibility was published previously[4]. Briefly, patients with an acute IS were consecutively enrolled at the University Hospital of Basel, within 72 hours of symptom onset. In the following validation set (study B), patients with IS were included within the first 6 hours after symptom onset, at the emergency department of the Vall d'Hebron University Hospital as published previously[9]. According to the World Health Organization criteria, acute IS was defined as an acute focal neurological deficit lasting >24 hours with no sign of intracranial hemorrhage on cerebral imaging[10]. Exclusion criteria in both studies were patients with hemorrhagic stroke, TIA and mimicking conditions, and missing informed consent. Furthermore, patients with history of and clinical signs of infection on admission or 14 days before stroke onset were not eligible. All patients received a blood and urine test as well as clinical examinations, at admission. Imaging examinations have been done at each clinical suspicion of any infection at admission. In both studies, mechanical ventilation was not an exclusion criterion. Comorbidities (including chronic inflammatory disease, cancer and renal impairment) were assessed by the modified Charlson Comorbidity Index[11]. A small number of patients received anti-inflammatory medication such as corticosteroids (n=11), but only 2 of them had an infection. Stroke etiology was defined according to the Trial of Org 10172 in Acute Stroke Treatment classification[12]. Stroke severity was assessed with the National Institutes of Health Stroke Scale (NIHSS)[13]. Supplementary, in study A, a subgroup of patients underwent magnetic resonance imaging, and assessment of lesion size was performed (n=135)[14]. The lesion size was calculated by experienced raters using a semiquantitative method as described[15], where

lesions were categorized into 3 size classes (small lesion, <10 cm^3; medium lesion, 10–100 cm^3; large lesion, >100 cm^3)[16]**.** In study B, trained nurses assessed dysphagia preferably within the first 24 hours after stroke onset, with the volume-viscosity swallow test.

2.3 Follow-Up and Outcome Measure

Primary outcome measure for this study was presence and time point of any poststroke infection throughout hospitalization. More precisely the timeline of observation was defined as the time from stroke onset for up to 5 days following the stroke for study A, and up to 7 days for study B. Infections were defined according to the US Centers for Disease Control and Prevention criteria 1988 to 2008 [17] for study A and the updated version 2008 to until now [18] for study B (at the time of enrollment for study A the updated Centers for Disease Control and Prevention criteria were not available). For both classification systems, blood biomarkers such as CRP or PCT are not required for diagnosis of infection.

The only difference between the older and new criteria is that radiological confirmation was mandatory to diagnose a pneumonia. For this study, all patients with any infection containing either respiratory tract infection, urinary tract infections, and other types of infections were included.[18] The treating physician diagnosed the infection during the hospitalization, according to the criteria mentioned above. A post-hoc validation was done using charts. Treating physicians were blinded to biomarker levels regarding the primary diagnosis, as well as the secondary validation. The onset of clinical symptoms led to further investigations and resulted in the diagnosis of infection. The time point of the diagnosis was referred to the beginning of antibiotic treatment.

2.4 Biomarker Measurement

Blood samples were collected at hospital admission before any treatment was administered.

Derivation study A: Blood samples were collected within 72 hours from symptom onset, including CRP, PCT, white blood cell count and SAA.

Validation Study B: Single blood sample was collected within 6 hours from symptom onset, including only SAA. In study B, conversely to study A, CRP, PCT, white blood cell, and monocytes were not measured.

SAA Measurement for Both Cohorts: After centrifugation during 15 minutes at 1500g at 4 °C, serum was obtained and stored at −80 °C until analysis. The Meso Scale Discovery Vascular Injury Panel-I ECL assay was used to determine the levels of SAA1/2, as per manufacturer's instructions (Meso Scale Discovery, Gaithersburg, MD). Dilution of 1:1000 was applied to each serum sample. Electrochemiluminescence detection system using multiarray technology (SECTOR Imager 2400, Meso Scale Discovery) was used to determine analyte concentrations. SAA determinations were performed blinded to clinical data. The coefficient of variation of the assay between duplicates was below 15%.

2.5 Statistical Analysis

Continuous variables were expressed as medians (interquartile range [IQR]) and categorical variables as counts (percentages). To test for normality, Shapiro-Wilk test was used. To achieve normality biomarker data were log-transformed. Pearson χ^2 test was used to compare categorical variables. Two-group comparison of continuous baseline data was performed by the Mann-Whitney *U* test.

To investigate the association of SAA with SAI, we calculated logistic regression models. Univariate analysis of infection was performed including variables that were significant in the derivation or validation cohort ($P<0.05$), and a first multivariate logistic regression model was constructed (model 1). Lesion size was not included in the whole model to avoid bias because in >50% of patients magnetic resonance imaging data were missing. Also, microangiopathic stroke was not included in the whole model, because there were no patients in the validation

cohort with infection and microangiopathic stroke. The nested model was the clinical model without the biomarker SAA, and the whole model included all predictors identified by the univariate regression analysis including the biomarker SAA. Additionally, to assess the independent association of SAA over additional laboratory predictors of infection collected just in one cohort (A), model 1 was further adjusted by conventional markers of infection (white blood cell, CRP, and PCT) in the derivation cohort (model 2) and by dysphagia (model 3). Furthermore, the association of SAA and lesion size was assessed by a binary regression model in a subgroup analysis of cohort A, where magnetic resonance imaging was performed (n=135; 47.7%). Receiver operating characteristic (ROC) curves along with the area under the ROC curve as an overall discriminatory measure were calculated. The likelihood ratio test was used to test the goodness-of-fit of the whole model versus the nested model. The net reclassification improvement (NRI) has been proposed to evaluate prognostic biomarkers[19]

3. Results

3.1 Baseline Data

Derivation Study A: Of 283 patients with acute IS, 60 (21.2%) developed an infection after onset of stroke during hospitalization. Twenty (7.1%) patients experienced any respiratory tract infection including pneumonia, 21 (7.4%) patients had urinary tract infections, and 19 (6.7%) patients had other infections (unknown, 1; sepsis, 11; phlebitis, 3; gastro- enteritis, 1, erysipel: 2; colpitis: 1). The median time of occurrence of any infection was 3 days after stroke (IQR 2-

7). The median age of the study cohort was 75 (IQR, 63–82) years, and 41% were women. Patient characteristics stratified by infected versus noninfected are summarized in Table 1.

Validation Study B: In the present study, 367 patients with acute IS were included; 111 (30.2%) of them developed an infection during hospital stay. Any infection of the respiratory tract occurred in 83 (22.6%) patients, including pneumonia (10.6%) and bronchitis (12%). Eighteen (5%) patients had urinary tract infections, and 22 (6%) had other infections. The median time of occurrence of any infection was 2 days after stroke (IQR 1-5). Patient characteristics stratified by infected versus noninfected are summarized in Table 1.

3.2 Prediction of poststroke Infection during Hospitalization

Derivation Study A: Patients with a higher SAA level (on day 0) were more prone to develop an infection (17 [IQR, 5–76] versus 5 [IQR, 3–13] μg/mL; P<0.001; Table 1). Patient's characteristics stratified by infection are shown in able 1. SAA was an independent predictor of poststroke infection when adjusted by these clinical variables (odds ratio [OR], 1.44 [95% CI, 1.16–1.79]; P=0.001; Table 2, model 1), and it contributed to an improved discriminatory accuracy of the whole multiple logistic regression model (area under the ROC curve change, from 0.76 [0.69–0.83] to 0.79 [0.72–0.86]; P<0.001; Table 3, model 1). The combination of SAA with the regression model also led to an increase in the continuous NRI of 46.9% (0.133–0.674). When the model was further adjusted by already known inflammatory markers such as white blood cell, CRP, and PCT, SAA remained as an independent predictor of post- stroke infection (OR, 1.45 [1.03–2.05]; P=0.034) and increased the NRI by 33.6% (−0.078 to 0.812; Table 2, model 2). In a subgroup analysis of patients with available lesion size (n=135; 47.7%), no change in the predictive value of SAA was observed (OR, 1.51 [1.13–2.01]; P=0.005; not shown in a table). An SAA cutoff level at 4.0 μg/mL gave a sensitivity of 81% and a specificity of 44.5% for infection during hospitalization.

Validation Study B: Compared with the derivation study, the validation study revealed similar results with higher SAA levels predicting the development of an infection (10.4 [5.3–55.5] versus 7.2 [3.7–18.7] µg/mL; P=0.0056; Table 1). The association could be shown even earlier, within 6 hours of stroke symptom onset. Patient's characteristics of the validation cohort were comparable to those of the derivation cohort. Patients with dysphagia had a higher risk for an infection (Table 1). The same logistic regression model (model 1) was evaluated in this validation cohort, and again SAA was independently associated with infection (OR, 1.52 [1.05–2.22]; P=0.028; Table 2, model 1). The improvement of the clinical model was statistically significant (area under the ROC curve change, from 0.75 [0.70– 0.81] to 0.76 [0.71–0.82]; P=0.038; Table 3, model 1).

4. Discussion

There is need for an accurate and simply available biomarker permitting early prediction of the development of infections after stroke, not only to prevent the development of infections but also for better therapy guidance. In a recently performed pilot study, SAA protein 1/2 was discovered as an early predictor of poststroke infection (SAI).[8] In the present study, we verified and validated SAA as a marker of SAI, in 2 independent cohorts of stroke patients, therefore, confirming and expanding the results of the previous discovery study. Due to the larger sample size of the current studies, adjustments for predictive factors were conceivable. Moreover the incremental value beyond demographic and inflammatory risk factors was evaluated using different statistical methods.

In fact, no biomarker has demonstrated usefulness neither in SAI diagnosis nor for its prediction, as reviewed by Smith et al[20]. The blood biomarker SAA is an acute-phase protein, which is upregulated by a variety of inflammatory stimuli[21]. In healthy individuals, SAA concentration in serum is around 1 to 5 µg/m [22]. However, during an acute-phase reaction the

concentration can rise to 1 mg/mL or even higher[23]. The function of SAA is still not well understood. It is known to have immunomodulatory activity[24, 25] and to be the only molecule that displays sensitivity and response speed (within hours) similar or even faster to those of CRP[26]. This is coherent with our observation. SAA levels measured on admission (even within 6 hours after stroke symptom onset) were significant predictors of any infection after stroke onset, before clinical signs of infection occurred and diagnostic work-up was initiated. In fact, SAA was an independent predictor of SAI and gave additional predictive value over clinical variables even when a powerful covariate like dysphagia was included in the model. Of note, SAA in comparison to dysphagia is an ultra-early predictor, allowing for risk stratification even before dysphagia can reliably be assessed. Moreover, this ultra-early prediction was not observed for CRP[27]. After adjusting for several predictive factors including inflammatory markers and severity of stroke, SAA remained independently associated with SAI suggesting an incremental prognostic value beyond these traditional risk factors. This incremental value was modest but significant shown by the improvement in discrimination when adding SAA to the best clinical model.

In a recently published proteomic discovery study, SAA was identified as a promising predictor marker of patients at risk of poststroke infection[4, 8, 27]. In a small (n=60) study, the highest SAA levels were observed between days 1 and 3 in stroke patients with infection compared with stroke patients without an infection[28]. study including 81 patients with subarachnoid hemorrhage, SAA levels were significantly higher in 54 patients who developed an infection in the course of their hospital stay than in 27 patients who did not[29]. In our study we could confirm the results of these pilot studies.

As an acute-phase inflammatory mediator, we can hypothesize that an early and severe inflammatory response in acute stroke, expressed by increased levels of SAA, might act as a predictor of the further development of SAI. Local inflammation is a part of the reactions after

stroke, and microglia activation occurs within hours after the ischemic event[30]. This local inflammatory response is rapidly extended to the peripheral circulation by the release of proinflammatory cytokines, which start the systemic inflammatory response by activating peripheral proteins acting as acute-phase reactants, such as SAA [31]. The amount of this inflammatory response has been related with a more severe immunodepression in the following days, conferring a high vulnerability for SAI[32, 33]. Therefore, our study supports the role of an early inflammatory marker, such as SAA, as a predictor of SAI. However, the precise mechanism underlying this association remains to be explored.

Given the high sensitivity, but lower specificity in a clinical setting where many clinicians start antibiotic treatment upon potentially misleading unspecific symptoms such as fever, the marker might be best used as "rule out" marker and as such it can help to avoid unnecessary antibiotic treatment in some patients with no bacterial infection, while still having a low risk of false negative results.

This seems particularly important regarding the selection of stroke patients for antibiotic treatment where empirical antibiotic prophylaxis is discouraged by current guidelines[34]. The combination of clinical variables as well as a panel of serum markers reflecting different pathways of the immunological and inflammatory response after stroke, such as SAA, IL-6, or classical markers such as CRP, might help to guide antibiotic treatment[35].

This study has limitations: First, different time-ranges for the measurements of SAA were used, so that the direct comparison of SAA between the studies was not possible. However, the first study was a proof of concept study to assess the magnitude of association in a wider time range and the second study was chosen to assess whether this association remained even within the very first hours after stroke where risk stratification should be ideally done to prevent infections, given that most of them occur within the first 48-72 hours after stroke. Second, no follow-up data on the detection of infection after hospitalization were available, thus potential

misclassification could have occurred. However, this potential misclassification would have led to a bias towards the null hypothesis and thus would rather lead to an underestimation of the predictive value of SAA. Third, since not all patients underwent an MRI we could not adjust for lesion size in all patients, which may contribute to infection prediction. In a subgroup analysis of patients with available lesion size (n=135) there was however no change in the predictive value suggesting no major confounding of the predictive value by lesion size. Fourth, as only patients with infections in the previous 14 days were excluded from the present study, we could not explore whether an infection before 14 days of stroke onset might have been responsible elevated SAA levels, as has been studied by Syrjänen et al[36]. Finally, although we adjusted for multiple potential confounders such as age, NIHSS score, hypertension, atrial fibrillation, dysphagia in one cohort, residual-confounding effects cannot be excluded.

In conclusion, among a total of 650 ischemic stroke patients, serum amyloid A measured at admission is a novel independent predictor of infection after stroke. SAA improved the prediction model of patients who developed any infection. In the future, if validated in a larger prospective cohort, SAA could be helpful for closely monitoring a subgroup of patients that should be further investigated in radiological or laboratory test in order to prevent the development of infection. SAA may facilitate the identification of patients who will not develop an infection in order to avoid unnecessary antibiotic treatment.

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Supplemental Material

Table I

Online Figures I-I

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Tables

Table 1

Patient Characteristics Stratified by Infectious Event

	Derivation Study A				**Validation Study B**			
	Total	**No infection**	**Infection**	***P-Value***	**Total**	**No infection**	**Infection**	***P-Value***
N (%)	283	223 (79)	60 (21)		367	256 (70)	111 (30)	
Demographic data								
Age, y, median (IQR)	75 (63-82)	75 (61-82)	79 (68-83)	0.12	79 (67-84)	77 (65-83)	82 (73-86)	<0.0001
Female sex, *n* (%)	115 (41)	82 (37)	33 (55)	0.012	192 (52)	136 (53)	56 (51)	0.651
Medical history, *n* (%) *								
Hypertension	213 (75)	159 (71)	54 (90)	0.007	264 (72)	175 (68)	89 (80)	0.039
Atrial fibrillation	57 (20)	38 (17)	19 (32)	0.018	151 (41)	88 (34)	63 (57)	<0.0001
Smoking	104 (37)	80 (36)	24 (40)	0.58	78 (21)	61 (24)	17 (15)	0.099
Diabetes mellitus	54 (19)	42 (19)	12 (20)	0.45	99 (27)	67 (26)	32 (29)	0.610
Coronary heart disease	61 (22)	50 (22)	11 (18)	0.76	67 (18)	43 (17)	24 (22)	0.304

Dyslipidaemia	73 (26)	59 (26)	14 (23)	0.29	178 (49)	122 (48)	56 (51)	0.572
Dysphagia	NA	NA	NA	NA	63 (17)	22 (9)	41 (37)	<0.0001
Thrombolysis and/or endovascular treatment	55 (19)	34 (15)	21 (35)	0.002	231 (63)	154 (60)	77 (69)	0.058
Modified Charlson Index, median (IQR)	1 (0-2)	1 (0-2)	1 (0-2)	0.100	NA	NA	NA	NA
Clinical data, median (IQR)								
Baseline NIHSS (points)	5 (2-10)	4 (2-8)	10 (5-17)	<0.0001	10 (6-17)	9 (5-14)	16 (9-20)	<0.0001
Laboratory values, median (IQR)								
White blood count (g/l)	8.2 (7-10)	7.8 (6-9)	9.6 (8-11)	<0.0001	NA	NA	NA	NA
Monocytes (%)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.27	NA	NA	NA	NA
C-reactive Protein (mg/l)	3 (3-8)	3 (3-6)	5 (3-17)	0.0008	NA	NA	NA	NA
Procalcitonin (pmol/l)	0.017 (0.012-0.025)	0.017 (0.012-0.024)	0.021 (0.014-0.33)	0.0047	NA	NA	NA	NA

SAA (µg/ml) †	6 (3-21)	5 (3-13)	17 (5-76)	<0.0001	7.8 (4-25)	7.2 (3.7-18.7)	10.4 (5.3-55.5)	0.0056
Stroke etiology (TOAST) , n (%)*								
Large vessel stroke	54 (19)	43 (19)	11 (18)	1.00	51 (14)	35 (14)	16 (14)	0.696
Cardioembolic stroke	97 (34)	68 (31)	29 (48)	0.02	166 (45)	106 (41)	60 (54)	0.063
Microangiopathic stroke	49 (17)	43 (19)	6 (10)	0.22	27 (7)	27 (11)	0	NA
Other known	13 (5)	11 (5)	2 (3)	0.80	11 (3)	8 (3)	3 (3)	1.00
Cryptogenic stroke	69 (24)	57 (26)	12 (20)	0.53	112 (31)	80 (31)	32 (29)	0.601

Abbreviations: y = years, IQR = interquartile range; NIHSS = National Institutes of Health Stroke Scale; SAA = Serum Amyloid A; CRP = C-reactive protein; WBC = white blood cells; PCT = Procalcitonin; TOAST = Trial of Org 10172 in Acute Stroke Treatment; NA = not applicable.

Statistics: Values are median (IQR) or number (percent). The *P-Values* were assessed using the Mann-Whitney U Test and Pearson chi-squared. *P-Values* <0.05 were considered statistically significant.

*Because of rounding, percentages may not total 100.

†SAA day 0: For study A the venous puncture was within 72h of stroke onset and in study B within 6h of stroke onset.

Table 2

Multivariable Logistic Regression Model For The Occurrence Of Any Post Stroke Infection

		Derivation Study A			**Validation Study B**		
Models	**Predictors**	**Odds Ratio**[a]	**95% - CI**	***P-Value***	**Odds Ratio**[a]	**95% - CI**	***P-Value***
	Log_SAA*	1.44	1.16 – 1.79	**0.001**	1.52	1.05 – 2.22	**0.028**
	Age	1.01	0.98 – 1.05	0.351	1.03	1.01– 1.05	**0.016**
	Female sex	1.80	0.88 – 3.69	0.110	0.59	0.35 – 1.01	0.054
Model 1	Baseline NIHSS	1.05	1.00 – 1.10	0.068	1.12	1.07 – 1.17	**<0.0001**
	Art. Hypertension	3.42	1.24 – 9.49	**0.018**	1.76	0.94 – 3.29	0.075
	Atrial fibrillation	1.02	0.38 – 2.74	0.963	2.07	0.94 – 4.63	0.076
	Cardioembolic stroke	1.31	0.60 – 2.93	0.504	0.66	0.30 – 1.46	0.308
	Thrombolysis and/or endovascular treatment	2.79	1.18 – 6.62	**0.020**	1.27	0.72 – 2.24	0.409
Pseudo-R2				0.1828			0.1596
Model 2	Log_SAA*	1.45	1.03 – 2.05	**0.034**	NA	NA	NA

	Age	1.03	0.99 – 1.06	0.113	NA	NA	NA
	Female sex	2.01	0.92 – 4.40	0.079	NA	NA	NA
	Baseline NIHSS	1.05	0.99 – 1.10	**0.084**	NA	NA	NA
	Art. Hypertension	2.73	0.96 – 7.74	**0.060**	NA	NA	NA
	Atrial fibrillation	0.74	0.26 – 2.15	0.583	NA	NA	NA
	Cardioembolic stroke	1.56	0.67 – 3.63	0.301	NA	NA	NA
	Thrombolysis and/or endovascular treatment	3.73	1.51 – 9.21	**0.004**	NA	NA	NA
	Log_WBC*	2.93	0.93 – 9.23	0.066	NA	NA	NA
	Log_CRP *	0.77	0.44 – 1.33	0.350	NA	NA	NA
	Log_PCT *	1.62	0.99 – 2.67	0.057	NA	NA	NA
Pseudo-R2				0.2303			NA
Model 3	Log_SAA*	NA	NA	NA	1.66	1.02 – 2.70	**0.040**
	Age	NA	NA	NA	1.01	0.98 – 1.04	0.389
	Female sex	NA	NA	NA	0.53	0.25 – 1.11	0.093
	Baseline NIHSS	NA	NA	NA	1.15	1.08 – 1.23	**<0.0001**

Art. Hypertension	NA	NA	NA	2.85	1.18 – 6.91	**0.020**
Atrial fibrillation	NA	NA	NA	1.07	0.36 – 3.17	0.901
Cardioembolic stroke	NA	NA	NA	0.81	0.29 – 1.61	0.389
Thrombolysis and/or endovascular treatment	NA	NA	NA	0.69	0.29 – 1.61	0.389
Dysphagia	NA	NA	NA	5.06	2.46 – 10.39	**<0.0001**
Pseudo-R2			NA			**0.2412**

Abbreviations: SAA = serum amyloid A; NIHSS = National Institutes of Health Stroke Scale; CRP = C-reactive protein; WBC = white blood cells; PCT = Procalcitonin; CI = confidence interval; NA = not applicable.

*Odds ratio refers to a 1-unit increase in the explanatory variable and to any 10-fold increase in SAA, WBC; CRP and WBC (log transformed with a base of 10). The odds ratio represents the change per some unit of SAA. All of the covariates entered in the models are listed in the table.

Table 3

AUC To Predict An Infectious Event During Hospitalization

	Derivation Study A			**Validation Study**		
Predictors	**AUC**	**95% - CI**	***Likelihood Ratio test*** [§]	**AUC**	**95% - CI**	***Likelihood Ratio test*** [§]
Model 1*	0.76	0.69 – 0.83	NA	0.75	0.70 – 0.81	NA
Model 1* and SAA	0.79	0.72 – 0.86	<0.0001	0.76	0.71 – 0.82	0.038
Model 2[†]	0.80	0.73 – 0.87	NA	NA	NA	NA
Model 2[†] and SAA	0.81	0.74 – 0.88	0.0314	NA	NA	NA
Model 3[‡]	NA	NA	NA	0.81	0.74 – 0.87	NA
Model 3[‡] and SAA	NA	NA	NA	0.81	0.76 – 0.88	0.0393

Abbreviations: SAA = serum amyloid A; NIHSS = National Institutes of Health Stroke Scale; CRP = C-reactive protein; WBC = white blood cells; PCT = Procalcitonin; NA = not applicable; AUC = Area under the curve.

[*]Model 1, (age, female sex, baseline NIHSS, hypertension, atrial fibrillation, cardioembolic stroke, Thrombolysis and/or endovascular treatment) multivariate logistic regression model presented in table 2.

[†]Model 2, (age, female sex, baseline NIHSS, hypertension, atrial fibrillation, cardioembolic stroke, Thrombolysis and/or endovascular treatment, WBC, CRP, PCT), multivariate logistic regression model presented in table 2.

[‡] Model 3, (age, female sex, baseline NIHSS, hypertension, atrial fibrillation, cardioembolic stroke, Thrombolysis and/or endovascular treatment, Dysphagia), multivariate logistic regression model presented in table 2.

[§] The likelihood ratio test was used to test the goodness-of-fit of the whole model vs the nested model.
